Data Evaluation Report on the Acute Toxicity of BAS 800 H (Saflufenacil) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547225 EPA MRID Number: 47127923

Data Requirement:	PMRA DATA CODE	9.8.2
	EPA DP Barcode	DP349851
	OECD Data Point	IIA 8.4
	EPA MRID	47127923
	EPA Guideline	OPPTS 850.5400

Test material: BAS 800 H **Purity:** 93.8%
Common name: Saflufenacil
Chemical name: IUPAC: Not Reported
CAS name: N'-[2-chloro-4-fluoro-5-(3-methyl-2,6-dioxo-4-(trifluoromethyl)-3,6-dihydro-1(2H)-pyrimidinyl)benzoyl]-N-isopropyl-N-methylsulfamide
CAS No.: 372137-35-4
Synonyms: None Reported

Primary Reviewer: John Marton **Signature:**
Staff Scientist, Cambridge Environmental, Inc. **Date:** 03/25/08

Secondary Reviewer: Teri S. Myers **Signature:**
Senior Scientist, Cambridge Environmental, Inc. **Date:** 04/08/08

Primary Reviewer: Anita Pease **Date:** 06/09/09
Senior Biologist, U.S. EPA

Secondary Reviewer: Ann Lee **Date:** 06/09/09
HC-PMRA-EAD

Secondary Reviewer: Farzad Jahromi **Date:** 06/09/09
DEWHA-APVMA

Company Code	BAZ
Active Code	SFF
Use Site Category:	13 (terrestrial feed crops) and 14 (terrestrial food crops)
EPA PC Code	118203

CITATION: Hoffman, F. 2006. Effect of BAS 800 H (Reg. No. 4054449) on the Growth of the Green Alga *Pseudokirchneriella subcapitata*. Unpublished study performed by BASF Aktiengesellschaft, BASF Agricultural Center Limburgerhof, Crop Protection Division, Ecology and Environmental Analytics, Limburgerhof, Germany. Laboratory report number 2007/7013577. Study sponsored by BASF Corporation, Agricultural Products Division, Research Triangle Park, NC. Study completed April 13, 2006; report amended November 12, 2007.

DISCLAIMER: This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to aquatic nonvascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of

factors related to the test methodology and results in determining the acceptability of the study.

Data Evaluation Report on the Acute Toxicity of BAS 800 H (Saflufenacil) to Algae (*Pseudokirchneriella subcapitata*)

PMRA Submission Number: 2008-0431
PMRA Document ID: 1547225 EPA MRID Number: 47127923

EXECUTIVE SUMMARY:

In a 96-hour acute toxicity study, cultures of the freshwater green alga *Pseudokirchneriella subcapitata* were exposed to BAS 800 H (Saflufenacil) at nominal concentrations of 0 (negative control), 0.020, 0.028, 0.039, 0.055, 0.077, 0.108, 0.151 and 0.211 mg a.i./L under static conditions; mean-measured concentrations were 0 (negative control), 0.0200, 0.0283, 0.0410, 0.0572, 0.0808, 0.114, 0.159 and 0.218 mg a.i./L. Cell count and yield were equally the most sensitive endpoints with NOAEC and EC_{50} values of <0.0200 and 0.042 mg a.i./L, respectively. EC_{05} values for cell count and yield were 0.015 and 0.016 mg a.i./L, respectively. The % growth inhibition, based on cell count, in the treated algal culture as compared to the control ranged from 5.6% to 99.6%.

No morphological effects were observed at nominal concentrations up to 0.077 mg a.i./L. At the nominal 0.108, 0.151 and 0.211 mg a.i./L treatment levels, cells appeared to thicker than control cells with the amount of thick cells increasing with higher concentrations.

This toxicity study is classified as **SUPPLEMENTAL** by the **U.S. EPA** and **FULLY RELIABLE** to **PMRA** and **APVMA**. The study is classified as SUPPLEMENTAL by the U.S. EPA because a definitive NOAEC value was not established. In order to satisfy the U.S. EPA's guideline requirement for an acute freshwater alga toxicity study, the study should be repeated with lower test concentrations such that no effects are observed at the lowest test concentration.

Results Synopsis

Test Organism: *Pseudokirchneriella subcapitata*
Test Type (Flow-through, Static, Static Renewal): Static

Cell Count:

EC_{05}:	0.015 mg a.i./L	95% C.I.: 0.014-0.017 mg a.i./L
EC_{10}:	0.019 mg a.i./L	95% C.I.: 0.017-0.021 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L
NOAEC:	<0.0200 mg a.i./L	
Probit Slope:	3.76±0.127	

Yield (0-96 Hours):

EC_{05}:	0.016 mg a.i./L	95% C.I.: 0.014-0.018 mg a.i./L
EC_{10}:	0.020 mg a.i./L	95% C.I.: 0.018-0.022 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L
NOAEC:	<0.0200 mg a.i./L	
Probit Slope:	3.82±0.129	

Growth Rate (0-96 Hours):

EC_{05}:	0.036 mg a.i./L	95% C.I.: 0.032-0.042 mg a.i./L
EC_{50}:	0.12 mg a.i./L	95% C.I.: 0.12-0.13 mg a.i./L
NOAEC:	0.0200 mg a.i./L	
Probit Slope:	3.13±0.142	

Endpoint(s) Affected: Cell Count, Yield, Growth Rate

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following guidelines outlined in OECD Guidelines for the Testing of Chemicals, 201, "Alga, Growth Inhibition Test." The following deviations from OPPTS 850.5400 were noted:

1. The size and fill volume of the test vessels were 100 and 60 mL, respectively. OPPTS guidance requires that the fill volume not exceed 50% of the size of the test vessel.
2. The source of the dilution water used to prepare the nutrient medium was not specified.
3. The results of a periodic screening analysis of the dilution water were not provided.
4. Due to significant treatment-related inhibitions at all levels, the reviewer was unable to determine NOAEC and EC_{05} values for cell count and yield, the most sensitive endpoints in this study.

The deviation associated with the lack of a definitive NOAEC value impacts the acceptability of the study for U.S. EPA. In order to satisfy the guideline requirement for an acute freshwater alga toxicity study, the study should be repeated with lower test concentrations such that no effects are observed at the lowest test concentration.

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the OECD Principles of Good Laboratory Practice and the GLP Principles of the German "Chemikaliengesetz" (Chemicals Act) and meets the United States Environmental Protection Agency Good Laboratory Practice Standards [40 CFR Part 160 (FIFRA) and Part 792 (TSCA)], with the exception that recognized differences existed between the GLP Principles/Standards of OECD and of FIFRA and TSCA.

A. MATERIALS:

1. Test material BAS 800 H (Saflufenacil)

Description: Solid White Powder

Lot No./Batch No. : COD-000515 (Batch Number)

Purity: 93.8%

Stability of compound under test conditions: The measured concentrations at test initiation yielded recoveries of 103.6-108.5% of nominal. Measured concentrations at test termination yielded recoveries of 95.5-104.6% of nominal and 91.8-98.3% of the initial measured concentrations.

(OECD recommends water solubility, stability in water and light, pKa, Pow, and vapor pressure of test compound)

Storage conditions of test chemicals: Stored at room temperature (5-25°C)

Physicochemical properties of BAS 800 H.

Parameter	Values	Comments
Water solubility at 20°C	0.21 mg/L*	BASF DocID 2005/ 7003391
Vapor pressure	4.5×10^{-15} Pa	20°C
UV absorption	272	pH1/pH7
pKa	Neutral	Ambient pH
Kow	Log P_{ow} 2.6	20°C

*** The study reported incorrect units for the water solubility. According to BASF DocID 2005/700391, the correct water solubility of saflufenacil at a pH of 7 and temperature of 20°C is 0.21 g/L.**

2. Test organism:

Name: Freshwater Green Alga (*Pseudokirchneriella subcapitata*)

EPA requires a nonvascular species: For tier I testing, only one species, S. capricornutum, to be tested; for tier II testing, S. costatum, A. flos-aquae, S. capricorntum, and a freshwater diatom is tested.

OECD suggests the following species are considered suitable: S. capricornutum, S. subspicatus, and C. vulgaris. If other species are used, the strain should be reported

Strain: Not Reported
Source: In-house laboratory cultures
Age of inoculum: 3 Days
Method of cultivation: Standard algal medium according to OECD Guideline 201

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: The study author reported the concentrations used for the definitive test were selected based on the results of a non-GLP range-finding test. The results from this range-finding test were not provided.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Acclimation period: Culturing media and conditions: (same as test or not) Health: (any mortality observed)	Continuous Same as test Not Reported	*EPA recommends two week acclimation period.* *OECD recommends an amount of algae suitable for the inoculation of test cultures and incubated under the conditions of the test and used when still exponentially growing, normally after an incubation period of about 3 days. When the algal cultures contain deformed or abnormal cells, they must be discarded.*
Test system Static/static renewal Renewal rate for static renewal	Static N/A	*EPA expects the test concentrations to be renewed every 3 to 4 days (one renewal for the 7 day test, 3-4 renewals for the 14 day test).*
Incubation facility	Temperature-controlled incubator	
Duration of the test	96 hours	*EPA requires: 96-120 hours* *OECD: 72 hours*
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	Glass 100 mL 60 mL	*OECD recommends 250 ml conical flasks are suitable when the volume of the test solution is 100 ml or use a culturing apparatus.*

Parameter	Details	Remarks *Criteria*
Details of growth medium name pH at test initiation: pH at test termination: Chelator used: Carbon source: Salinity (**for marine algae**):	Standard OECD Algal Medium 8.1 7.73-7.94 Yes $NaHCO_3$ N/A	The range of pH values at test termination represent the range of the treatment means. *OECD recommends the medium pH after equilibration with air is ~8 with less than .001 mmol/l of chelator if used.* *EPA recommends 20X-AAP and chelating agents (e.g. EDTA) in the nutrient medium for optimum cell growth. Lower concentrations of chelating agents (down to one-third of the normal concentration recommended for AAP medium) may be used in the nutrient medium used for test solution preparation if it is suspected that the chelator will interact with the test material. ASTM reference, E1415-91and D 3978-80 (reapproved 1987).*
If non-standard nutrient medium was used, detailed composition provided (Yes/No)	Yes	
Dilution water source/type: pH: salinity (**for marine algae**): water pretreatment (if any): Total Organic Carbon: particulate matter: metals: pesticides: chlorine:	 Not Specified Adjusted to 8.1 N/A Filter-Sterilized Not Reported Not Reported Not Reported Not Reported Not Reported	*EPA pH: Skeletonema costatum= ~8.0 Others = ~7.5 from beginning to end of the test. EPA salinity: 30-35 ppt. EPA is against the use of dechlorinated water.* *OECD: pH is measured at beginning of the test and at 72 hours, it should not normally deviate by more than one unit during the test.*
Indicate how the test material is added to the medium (added directly or used stock solution)	Serial dilution of a stock solution	

Parameter	Details	Remarks *Criteria*
Aeration or agitation	Agitation; approximately 135 rpm	
Initial cells density	$3x10^3$ cells/mL	*EPA requires an initial number of 3,000 - 10,000 cells/mL. For Anabaena flos-aquae, cell counts on day 2 are not required.* *OECD recommends that the initial cell concentration be approximately 10,000 cells/ml for S. capricornutum and S. subspicatus. When other species are used the biomass should be comparable.*
Number of replicates Control: Solvent control: Treatments:	 10 N/A 5/level	*EPA requires a negative and/or solvent 1xcontrol with 3 or more replicates per doses. Navicula sp.tests should be conducted with four replicate.* *OECD preferably three replicates at each test concentration and ideally twice that number of controls. When a vehicle is used to solubilize the test substance, additional controls containing the vehicle at the highest concentration used in the test.*
Test concentrations		

Parameter	Details	Remarks *Criteria*
Nominal: Measured:	0 (negative control), 0.020, 0.028, 0.039, 0.055, 0.077, 0.108, 0.151 and 0.211 mg a.i./L 0 (negative control), 0.0200, 0.0283, 0.0410, 0.0572, 0.0808, 0.114, 0.159 and 0.218 mg a.i./L	*EPA requires at least 5 test concentrations, with each at least 60% of the next higher one.* *OECD recommends at least five concentrations arranged in a geometric series, with the lowest concentration tested should have no observed effect on the growth of the algae. The highest concentration tested should inhibit growth by at least 50% relatively to the control and, preferably, stop growth completely.*
Solvent (type, percentage, if used)	N/A; a solvent was not used	
Method and interval of analytical verification	Samples were collected on Days 0 and 4 and were analyzed using HPLC with MS-detection.	
Test conditions Temperature: Photoperiod: Light intensity and quality:	22±1°C Continuous uniform illumination Universal white-type fluorescent lamps provided a light intensity of approximately 8000 lux.	*EPA temperature: Skeletonema: 20EC, Others: 24-25EC; EPA photoperiod: S. costatum 14 hr light/ 10 hr dark, Others: Continuous; EPA light: Anabaena: 2.0 Klux (±15%), Others: 4 - 5 Klux (±15%)* *OECD recommended the temperature in the range of 21 to25°C maintained at ± 2°C and continuous uniform illumination provided at approximately 8000 Lux measured with a spherical collector.*
Reference chemical (if used) name: concentrations:	Potassium dichromate Not Specified	The study with the reference item was conducted in December 2005 under Laboratory Study Code 235834. The 72-hour EC_{50} values for growth rate and yield were 1.05 and 0.38 mg/L, respectively.

Parameter	Details	Remarks *Criteria*
Other parameters, if any	None	

2. Observations:

Table 2: Observation parameters

Parameters	Details	Remarks Criteria
Parameters measured including the growth inhibition/other toxicity symptoms	-Cell count -Yield -Growth rate -Morphological observations	Yield was defined as the biomass at test termination minus the biomass at test initiation. *EPA recommends the growth of the algae expressed as the cell count per mL, biomass per volume, or degree of growth as determined by spectrophotometric means.*
Measurement technique for cell density and other end points	Cell count was determined using a spectrophotometer (wavelength 623 nm, 5 cm glass cuvettes; a 1 cm glass cuvette was used at 96 hours due to high cell density). Growth rate and yield were determined using the cell counts. Morphological observations were made using a microscope.	*EPA recommends the measurement technique of cell counts or chlorophyll a* *OECD recommends the electronic particle counter, microscope with counting chamber, fluorimeter, spectrophotometer, and colorimeter. (note: in order to provide useful measurements at low cell concentrations when using a spectrophotometer, it may be necessary to use cuvettes with a light path of at least 4 cm).*
Observation intervals	Cell counts were made every 24 hours. Yield was determined at 72 and 96 hours. Growth rate was determined for every 24-hour interval and for 0-72 and 0-96 hours.	*EPA and OECD: every 24 hours.*
Other observations, if any	None reported	
	By test termination, mean cell	

Parameters	Details	Remarks Criteria
Indicate whether there was an exponential growth in the control	count was $2.038x10^6$ cells/mL in the negative control, which is more than 2X of initial ($3x10^3$ cells/mL).	*EPA requires control cell count at termination to be 2X initial count or by a factor of at least 16 during the test.* *OECD: cell concentration in control cultures should have increased by a factor of at least 16 within three days.*
Were raw data included?	Yes	

II. RESULTS and DISCUSSION:

A. INHIBITORY EFFECTS:

Following 96 hours of exposure, the mean cell count was $20.38x10^5$ cells/mL in the negative control and 19.24, 13.53, 11.87, 6.66, 2.24, 1.04, 0.463 and $0.089x10^5$ cells/mL in the mean-measured 0.0200, 0.0283, 0.0410, 0.0572, 0.0808, 0.114, 0.159 and 0.218 mg a.i./L treatment groups, respectively, yielding inhibitions of 5.6, 33.6, 41.8, 67.3, 89.0, 94.9, 97.7 and 99.6%, respectively, relative to the negative control. The study author did not statistically analyze cell count.

By test termination, yield was $20.35x10^5$ cells/mL in the negative control and 19.21, 13.50, 11.84, 6.63, 2.21, 1.007, 0.433 and $0.059x10^5$ cells/mL in the mean-measured 0.0200, 0.0283, 0.0410, 0.0572, 0.0808, 0.114, 0.159 and 0.218 mg a.i./L treatment groups, respectively, yielding inhibitions of 5.6, 33.7, 41.8, 67.4, 89.1, 95.1, 97.9 and 99.7%, respectively, relative to the negative control. The 96-hours EC_{10} and EC_{50} values (determined by the study author) were 0.020 and 0.041 mg a.i./L, respectively, based on the nominal concentrations.

The 0-96 hour growth rate averaged 1.630 in the negative control and 1.616, 1.528, 1.494, 1.350, 1.077, 0.883, 0.679 and 0.272 in the mean-measured 0.0200, 0.0283, 0.0410, 0.0572, 0.0808, 0.114, 0.159 and 0.218 mg a.i./L treatment groups, respectively, yielding inhibitions of 0.9, 6.3, 8.3, 17.2, 33.9, 45.8, 58.3 and 83.3%, respectively, relative to the negative control. The 96-hours EC_{10} and EC_{50} values (determined by the study author) were 0.041 and 0.113 mg a.i./L, respectively, based on the nominal concentrations.

No morphological effects were observed at nominal concentrations up to 0.77 mg a.i./L. At the nominal 0.108, 0.151 and 0.211 mg a.i./L treatment levels, cells appeared to thicker than control cells with the amount of thick cells increasing with increasing concentration.

Table 3: Effect of BAS 800 H on algal growth, freshwater green alga (*Pseudokirchneriella subcapitata*)

Mean-Measured and (Nominal) Concentrations mg a.i./L	Initial Cell Density x10^5 cells/mL	Cell Density (x10^5 cells/mL) at			
		48 hours	72 Hours	96 Hours	
				Cell Count	% Inhibition[1]
Negative control	0.03	0.9953	5.349	20.38	N/A
0.0200 (0.020)	0.03	1.010	5.230	19.24	5.6
0.0283 (0.028)	0.03	0.8152	3.719	13.53	33.6
0.0410 (0.039)	0.03	0.5989	2.911	11.87	41.8
0.0572 (0.055)	0.03	0.2973	1.859	6.656	67.3
0.0808 (0.077)	0.03	0.2466	0.6844	2.238	89.0
0.114 (0.108)	0.03	0.1692	0.5562	1.037	94.9
0.159 (0.151)	0.03	0.09177	0.3373	0.4628	97.7
0.218 (0.211)	0.03	0.01970	0.05707	0.08910	99.6
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

N/A- Not Applicable
[1] Inhibitions were reviewer-calculated

Table 4: Effect of BAS 800 H on algal growth, freshwater green alga (*Pseudokirchneriella subcapitata*)

Mean-Measured and (Nominal) Concentrations mg a.i./L	Initial Cell Density x10^5 cells/mL	Yield- 96 Hrs (x10^5 cells/mL)	Yield % Inhibition[1]	Growth Rate 0-96h	Growth Rate Inhibition[1]
Negative control	0.03	20.35	N/A	1.630	N/A
0.0200 (0.020)	0.03	19.21	5.6	1.616	0.9
0.0283 (0.028)	0.03	13.50	33.7	1.528	6.3
0.0410 (0.039)	0.03	11.84	41.8	1.494	8.3
0.0572 (0.055)	0.03	6.626	67.4	1.350	17.2
0.0808 (0.077)	0.03	2.208	89.1	1.077	33.9
0.114 (0.108)	0.03	1.007	95.1	0.883	45.8
0.159 (0.151)	0.03	0.4328	97.9	0.679	58.3
0.218 (0.211)	0.03	0.0591	99.7	0.272	83.3
Reference chemical (if used)	N/A	N/A	N/A	N/A	N/A

7N/A- Not Applicable
[1] Inhibitions were reviewer-calculated

Table 5: Statistical endpoint values after 96 hours.

Statistical Endpoint	Cell Count	Yield	Growth Rate
NOAEC or EC_{05} (mg a.i./L)	N.D.	Not reported	Not reported
EC_{50} (mg a.i./L)	N.D.	0.041	0.113
IC_{50} or EC_{50} (mg a.i./L) (95% C.I.)	N.D.	0.041-0.042	0.110-0.116
Other (IC_{10}/EC_{10})	N.D.	0.020 (0.019-0.021)	0.041 (0.039-0.042)
Reference chemical[1], if used NOAEC IC_{50}/EC_{50}	N/A	0.38 (0.36-0.39)	1.05 (1.00-1.10)

N.D.- Not Determined
N/A- Not Applicable
[1] EC_{50} values for the reference chemical, potassium dichromate, were reported based on observations after 72 hours.

B. REPORTED STATISTICS:

The mathematical determinations of the ECx values for growth rate and yield were done by probit analysis. The calculations were conducted with a PC and the commercial software "TOXSTAT 3.5." All analyses were conducted using the nominal concentrations.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Methods: Prior to determining the toxicity values for cell count, yield and growth rate, the reviewer tested each data set for normality using the Chi-square and Shapiro-Wilks tests and for homogeneity of variance using the Hartley and Bartlett tests. If the data met these assumptions of ANOVA, the reviewer determined the NOAEC value using the parametric Bonferroni and Williams tests. If the data did not meet these assumptions, the NOAEC value was determined using the non-parametric Kruskal-Wallis test and direct observation of the dose-response data. Tests for normality and homogeneity and NOAEC determinations were made using Toxstat statistical software. The ECx values, 95% C.I. and slopes were estimated using the probit analysis via Nuthatch statistical software. All analyses were conducted using the mean-measured concentrations.

Cell Count:

EC_{05}:	0.015 mg a.i./L	95% C.I.: 0.014-0.017 mg a.i./L
EC_{10}:	0.019 mg a.i./L	95% C.I.: 0.017-0.021 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L
NOAEC:	<0.0200 mg a.i./L	
Probit Slope:	3.76±0.127	

Yield (0-96 Hours):

EC_{05}:	0.016 mg a.i./L	95% C.I.: 0.014-0.018 mg a.i./L
EC_{10}:	0.020 mg a.i./L	95% C.I.: 0.018-0.022 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L
NOAEC:	<0.0200 mg a.i./L	
Probit Slope:	3.82±0.129	

Growth Rate (0-96 Hours):

EC_{05}:	0.036 mg a.i./L	95% C.I.: 0.032-0.042 mg a.i./L
EC_{50}:	0.12 mg a.i./L	95% C.I.: 0.12-0.13 mg a.i./L
NOAEC:	0.0200 mg a.i./L	
Probit Slope:	3.13±0.142	

D. STUDY DEFICIENCIES:

A NOAEC value could not be determined for the most sensitive endpoints (cell count and 0-96 hr yield), due to significant inhibition at all treatment levels.

E. REVIEWER'S COMMENTS:

The reviewer's results were based on the mean-measured concentrations, while those of the study author were based on the nominal concentrations. Therefore, the reviewer's results are reported in the Executive Summary and

Conclusions sections of this DER.

The reviewer's non-parametric analysis for cell count and yield indicated NOAEC values of 0.0572 mg a.i./L; however, William's test (run during the probit analysis) indicated that all treatment levels were significantly different from the control for both endpoints. Furthermore, probit analysis yielded respective EC_{05} values of 0.015 and 0.016 mg a.i./L (less than the lowest treatment level) for cell count and yield, indicating that the test organism was highly sensitive to the test material at all mean-measured concentrations tested. Therefore, the reviewer visually determined the NOAEC values for these endpoints to be <0.0200 mg a.i./L. William's test also indicated that the growth rate was significantly inhibited at the mean-measured 0.0283-0.218 mg a.i./L treatment levels, yielding a NOAEC value of 0.0200 mg a.i./L. This NOAEC value was more conservative than the value generated by the reviewer's non-parametric analysis (0.0572 mg a.i./L). Therefore, the reviewer reported that the NOAEC value for growth rate was 0.0200 mg a.i./L.

The in-life portion of the definitive toxicity test was conducted from January 15 to January 20, 2006.

F. CONCLUSIONS:

This toxicity study is classified as SUPPLEMENTAL by the U.S. EPA and FULLY RELIABLE to PMRA and APVMA. The study is classified as SUPPLEMENTAL by the U.S. EPA because a definitive NOAEC value was not established. Cell count and yield were the most sensitive endpoints with NOAEC and EC_{50} values of <0.0200 and 0.042 mg a.i./L, respectively. EC_{05} values for cell count and yield were 0.015 and 0.016 mg a.i./L, respectively.

Cell Count:

EC_{05}:	0.015 mg a.i./L	95% C.I.: 0.014-0.017 mg a.i./L
EC_{10}:	0.019 mg a.i./L	95% C.I.: 0.017-0.021 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L

NOAEC: <0.0200 mg a.i./L
Probit Slope: 3.76±0.127

Yield (0-96 Hours):

EC_{05}:	0.016 mg a.i./L	95% C.I.: 0.014-0.018 mg a.i./L
EC_{10}:	0.020 mg a.i./L	95% C.I.: 0.018-0.022 mg a.i./L
EC_{50}:	0.042 mg a.i./L	95% C.I.: 0.040-0.045 mg a.i./L

NOAEC: <0.0200 mg a.i./L
Probit Slope: 3.82±0.129

Growth Rate (0-96 Hours):

EC_{05}:	0.036 mg a.i./L	95% C.I.: 0.032-0.042 mg a.i./L
EC_{50}:	0.12 mg a.i./L	95% C.I.: 0.12-0.13 mg a.i./L

NOAEC: 0.0200 mg a.i./L
Probit Slope: 3.13±0.142
Endpoint(s) Affected: Cell Count, Yield, Growth Rate

III. REFERENCES:

No references were provided.

APPENDIX I. OUTPUT OF REVIEWER'S STATISTICAL VERIFICATION:

```
Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cd       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
--------------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     _____

EXPECTED     3.283        11.858            18.718          11.858       3.283
OBSERVED     0            17                14              16           2

--------------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   8.6501
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cd       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =   13.051

W =    0.882

Critical W (P = 0.05) (n = 49) = 0.947
Critical W (P = 0.01) (n = 49) = 0.929
--------------------------------------------------------------------------------

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.


Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cd       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

--------------------------------------------------------------------------------

Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
```

File: 7923cd Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	20.383	20.383	445.000
2	0.0200	19.242	19.242	185.000
3	0.0283	13.532	13.532	157.000
4	0.0410	11.876	11.876	138.000
5	0.0572	6.656	6.656	110.000
6	0.0808	2.238	2.238	85.000
7	0.114	1.037	1.037	60.000
8	0.159	0.463	0.463	35.000
9	0.218	0.089	0.089	10.000

Calculated H Value = 46.704 Critical H Value Table = 15.510
Since Calc H > Crit H REJECT Ho:All groups are equal.

Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cd Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 9	8	7	6	5	4	3	2	1
9	0.218	0.089	0.089	\								
8	0.159	0.463	0.463	.	\							
7	0.114	1.037	1.037	.	.	\						
6	0.0808	2.238	2.238	.	.	.	\					
5	0.0572	6.656	6.656	.	.	.	.	\				
4	0.0410	11.876	11.876	.	.	.	.	.	\			
3	0.0283	13.532	13.532	.	.	.	.	.	.	\		
2	0.0200	19.242	19.242	*	*	.	.	.	.	.	\	
1	neg control	20.383	20.383	*	*	*	*	.	.	.	.	\

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,9) = 3.197 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.015	0.014	0.017	0.025	0.89
EC10	0.019	0.017	0.021	0.022	0.90
EC25	0.028	0.026	0.030	0.017	0.92
EC50	0.042	0.040	0.045	0.012	0.95

Slope = 3.76 Std.Err. = 0.127

```
!!!Poor fit: p < 0.001 based on DF=     6.00     40.0
-------------------------------------------------------------------------
7923CD : Cell count (x10^5 cells/mL), 96 hours; mg a.i./L
-------------------------------------------------------------------------
Observed vs. Predicted Treatment Group Means
-------------------------------------------------------------------------
     Dose      #Reps.      Obs.      Pred.       Obs.      Pred.    %Change
                           Mean      Mean      -Pred.    %Control

     0.00       10.0      20.4      20.5     -0.132       100.       0.00
   0.0200       5.00      19.2      18.2       1.01       88.9       11.1
   0.0283       5.00      13.5      15.2      -1.71       74.3       25.7
   0.0410       5.00      11.9      10.6       1.24       51.9       48.1
   0.0572       5.00      6.66      6.35      0.306       31.0       69.0
   0.0808       5.00      2.24      2.96     -0.722       14.4       85.6
    0.114       5.00      1.04      1.07    -0.0352       5.23       94.8
    0.159       5.00     0.463     0.310      0.152       1.51       98.5
    0.218       4.00    0.0891    0.0751     0.0140      0.366       99.6

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

Yield (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cy       Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies
-------------------------------------------------------------------------

INTERVAL     <-1.5     -1.5 to <-0.5     -0.5 to 0.5     >0.5 to 1.5     >1.5
             _____     _____________     ___________     ___________     ____

EXPECTED     3.283        11.858           18.718          11.858       3.283
OBSERVED     0            17               14              16           2

-------------------------------------------------------------------------
Calculated Chi-Square goodness of fit test statistic =   8.6501
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


Yield (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cy       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
-------------------------------------------------------------------------

D =   13.051

W =    0.882

Critical W (P = 0.05) (n = 49) = 0.947
Critical W (P = 0.01) (n = 49) = 0.929
-------------------------------------------------------------------------

Data FAIL normality test. Try another transformation.
```

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Yield (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cy Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Yield (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cy Transform: NO TRANSFORMATION

KRUSKAL-WALLIS ANOVA BY RANKS - TABLE 1 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	RANK SUM
1	neg control	20.353	20.353	445.000
2	0.0200	19.212	19.212	185.000
3	0.0283	13.502	13.502	157.000
4	0.0410	11.846	11.846	138.000
5	0.0572	6.626	6.626	110.000
6	0.0808	2.208	2.208	85.000
7	0.114	1.007	1.007	60.000
8	0.159	0.433	0.433	35.000
9	0.218	0.059	0.059	10.000

Calculated H Value = 46.704 Critical H Value Table = 15.510
Since Calc H > Crit H REJECT Ho:All groups are equal.

Yield (x10^5 cells/mL), 96 hours; mg a.i./L
File: 7923cy Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 0 0 9 8 7 6 5 4 3 2 1
9	0.218	0.059	0.059	\
8	0.159	0.433	0.433	. \
7	0.114	1.007	1.007	. . \
6	0.0808	2.208	2.208	. . . \

5	0.0572	6.626	6.626	. . . . \
4	0.0410	11.846	11.846	. . . . . \
3	0.0283	13.502	13.502	. . . . . \
2	0.0200	19.212	19.212	* * . . . . . \
1	neg control	20.353	20.353	* * * * . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,9) = 3.197 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	95% Bounds Upper	Std.Err.	Lower Bound /Estimate
EC5	0.016	0.014	0.018	0.024	0.89
EC10	0.020	0.018	0.022	0.021	0.91
EC25	0.028	0.026	0.030	0.017	0.93
EC50	0.042	0.040	0.045	0.012	0.95

Slope = 3.82 Std.Err. = 0.129

!!!Poor fit: p < 0.001 based on DF= 6.00 40.0

7923CY : Yield (x10^5 cells/mL), 96 hours; mg a.i./L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	10.0	20.4	20.4	-0.0894	100.	0.00
0.0200	5.00	19.2	18.2	0.963	89.3	10.7
0.0283	5.00	13.5	15.3	-1.77	74.7	25.3
0.0410	5.00	11.8	10.6	1.21	52.0	48.0
0.0572	5.00	6.63	6.30	0.328	30.8	69.2
0.0808	5.00	2.21	2.89	-0.682	14.1	85.9
0.114	5.00	1.01	1.02	-0.0151	5.00	95.0
0.159	5.00	0.433	0.287	0.146	1.40	98.6
0.218	4.00	0.0591	0.0667	-0.00764	0.326	99.7

!!!Warning: EC5 not bracketed by doses evaluated.

!!!Warning: EC10 not bracketed by doses evaluated.

Growth rate, 0-96 hours; mg a.i./L
File: 7923gr Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	3.283	11.858	18.718	11.858	3.283
OBSERVED	0	17	14	16	2

```
Calculated Chi-Square goodness of fit test statistic =   8.6501
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.


Growth rate, 0-96 hours; mg a.i./L
File: 7923gr       Transform: NO TRANSFORMATION

Shapiro Wilks test for normality
--------------------------------------------------------------------------------

D =    0.029

W =    0.911

Critical W (P = 0.05) (n = 49) = 0.947
Critical W (P = 0.01) (n = 49) = 0.929
--------------------------------------------------------------------------------

Data FAIL normality test. Try another transformation.

Warning -  The two homogeneity tests are sensitive to non-normal data and
           should not be performed.


Growth rate, 0-96 hours; mg a.i./L
File: 7923gr       Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance
--------------------------------------------------------------------------------

These two tests can not be performed because at least one group has
zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

--------------------------------------------------------------------------------

Growth rate, 0-96 hours; mg a.i./L
File: 7923gr       Transform: NO TRANSFORMATION

              KRUSKAL-WALLIS ANOVA BY RANKS  -  TABLE 1 OF 2
--------------------------------------------------------------------------------
                                TRANSFORMED   MEAN CALCULATED IN      RANK
GROUP    IDENTIFICATION           MEAN         ORIGINAL UNITS         SUM
-----  --------------------    -----------   ------------------  -----------
  1           neg control        1.630             1.630          445.000
  2                0.0200        1.616             1.616          185.000
  3                0.0283        1.527             1.527          157.000
  4                0.0410        1.493             1.493          138.000
  5                0.0572        1.350             1.350          110.000
  6                0.0808        1.077             1.077           85.000
  7                 0.114        0.883             0.883           60.000
```

8	0.159	0.679	0.679	35.000
9	0.218	0.272	0.272	10.000

Calculated H Value = 46.704 Critical H Value Table = 15.510
Since Calc H > Crit H REJECT Ho:All groups are equal.

Growth rate, 0-96 hours; mg a.i./L
File: 7923gr Transform: NO TRANSFORMATION

DUNNS MULTIPLE COMPARISON - KRUSKAL-WALLIS - TABLE 2 OF 2

GROUP	IDENTIFICATION	TRANSFORMED MEAN	ORIGINAL MEAN	GROUP 0 0 0 0 0 0 0 0 0 9 8 7 6 5 4 3 2 1
9	0.218	0.272	0.272	\
8	0.159	0.679	0.679	. \
7	0.114	0.883	0.883	. . \
6	0.0808	1.077	1.077	. . . \
5	0.0572	1.350	1.350	. . . . \
4	0.0410	1.493	1.493	. . . . . \
3	0.0283	1.527	1.527	. . . . . \
2	0.0200	1.616	1.616	* * . . . . . \
1	neg control	1.630	1.630	* * * * . . . . \

* = significant difference (p=0.05) . = no significant difference
Table q value (0.05,9) = 3.197 Unequal reps - multiple SE values

Estimates of EC%

Parameter	Estimate	95% Bounds Lower	Upper	Std.Err.	Lower Bound /Estimate
EC5	0.036	0.032	0.042	0.030	0.87
EC10	0.047	0.042	0.053	0.025	0.89
EC25	0.074	0.069	0.080	0.017	0.92
EC50	0.12	0.12	0.13	0.010	0.95

Slope = 3.13 Std.Err. = 0.142

!!!Poor fit: p < 0.001 based on DF= 6.00 40.0

7923GR : Growth rate, 0-96 hours; mg a.i./L

Observed vs. Predicted Treatment Group Means

Dose	#Reps.	Obs. Mean	Pred. Mean	Obs. -Pred.	Pred. %Control	%Change
0.00	10.0	1.63	1.61	0.0228	100.	0.00
0.0200	5.00	1.62	1.60	0.0196	99.3	0.702
0.0283	5.00	1.53	1.57	-0.0421	97.6	2.36
0.0410	5.00	1.49	1.50	-0.00267	93.1	6.93
0.0572	5.00	1.35	1.36	-0.0131	84.8	15.2

0.0808	5.00	1.08	1.14	-0.0682	71.2	28.8
0.114	5.00	0.883	0.863	0.0198	53.7	46.3
0.159	5.00	0.679	0.578	0.101	36.0	64.0
0.218	4.00	0.272	0.346	-0.0741	21.5	78.5